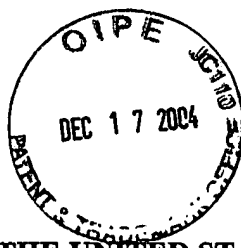


S/N 09/645706

PATENT



**IN THE UNITED STATES PATENT AND TRADEMARK OFFICE**

Applicant:	Keith V. Wood et al.	Examiner:	Rebecca E. Prouty
Serial No.:	09/645706	Group Art Unit:	1652
Filed:	August 24, 2000	Docket No.:	341.005US1
Title:	SYNTHETIC NUCLEIC ACID MOLECULE COMPOSITIONS AND METHODS OF PREPARATION		

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**DECLARATION UNDER 37 C.F.R. § 1.132**

Commissioner for Patents  
Washington, D.C. 20231

Sir:

I, Monika Wood, M.S., declare and say as follows:

1. I am one of the named co-inventors of the claims in the above-identified application. I make this Declaration in support of the patentability of the claims of the above-identified application.
2. Codon replacements in a nucleic acid sequence in the absence of affirmative selection of codons to reduce the introduction of potential regulatory sites will likely result in a synthetic nucleic acid sequence with additional potential regulatory sites including potential transcription factor binding sites.
3. For instance, using parameters similar to those employed in the above-referenced application, transcription factor binding sites were identified in the parent *gfp* gene (*gfp10*) and corresponding human codon optimized gene (*gfph*) in Zolotukhin et al. (U.S. Patent No. 5,874,304). For my analysis, I used TESS version 2.0 and TRANSFAC version 3.2.
4. Using those parameters, I found that the *gfp10* gene contained 133 transcription factor binding sites. After codon optimization, the resulting gene had 150 transcription factor binding sites-an increase of almost 20 new sites.
5. I further declare that all statements made herein of my own knowledge are true, and that all statements made on information and belief are believed to be true; and further that these statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under Section 1001 of Title

DECLARATION UNDER 37 CFR § 1.132

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Serial Number: 09/156,946 D.t.: 341.006US1

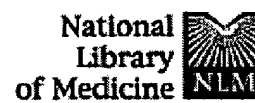
Filing Date: September 18, 1998

Title: THERMOSTABLE LUCIFERASES AND METHODS OF PRODUCTION

18 of the United States Code, and that such willful false statements may jeopardize the validity of the application or any patent issued thereon.

Dated: 12-09-2004

By: Monika Wood  
Monika Wood



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☐ 1: J Mol Biol. 1995 Dec 15;254(5):993-1005.

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## Steroid recognition by chloramphenicol acetyltransferase: engineering and structural analysis of a high affinity fusidic acid binding site.

Murray IA, Cann PA, Day PJ, Derrick JP, Sutcliffe MJ, Shaw WV, Leslie AG.

Department of Biochemistry, University of Leicester, UK.

The antibiotic fusidic acid and certain closely related steroidal compounds are potent competitive inhibitors of the type I variant of chloramphenicol acetyltransferase (CATI). In the absence of crystallographic data for CATI, the structural determinants of steroid binding were identified by (1) construction *in vitro* of genes encoding chimaeric enzymes containing segments of CATI and related type III variant (CATIII) and (2) site-directed mutagenesis of the gene encoding CATIII, followed by kinetic characterisation of the substituted variants. Replacement of four residues of CATIII (Gln92, Asn146, Tyr168 and Ile172) with their equivalents from CATI yields an enzyme variant that is susceptible to competitive inhibition by fusidate with respect to chloramphenicol ( $K_i = 5.4 \mu\text{M}$ ). The structure of the complex of fusidate and the Q92C/N146F/Y168F/I172V variant, determined at 2.2 Å resolution by X-ray crystallography, reveals the inhibitor bound deep within the chloramphenicol binding site and in close proximity to the side-chain of His195, an essential catalytic residue. The aromatic side-chain of Phe146 provides a critical hydrophobic surface which interacts with non-polar substituents of the steroid; the remaining three substitutions act in concert both to maintain the appropriate orientation of Phe 146 and via additional interactions with the bound inhibitor. Substitution of Gln92 by Cys eliminates a critical hydrogen bond interaction which constrains a surface loop (residues 137 to 142) of wild-type CATIII which must move in order for fusidate to bind to the enzyme. Only two hydrogen bonds are observed in the CAT-fusidate complex, involving the 3- $\alpha$ -hydroxyl of the steroid ring and both hydroxyl of Tyr25 and NE2 of His195, both of which are also involved in hydrogen bonds with substrate in the CATIII-chloramphenicol complex. In the acetyl transfer reaction catalysed by CAT, NE2, of His195 serves as a general base in the abstraction of a proton from the 3-hydroxyl of chloramphenicol as the first chemical step in catalysis. The structure of the CAT-inhibitor complex suggests that deprotonation of the 3- $\alpha$ -hydroxyl of both fusidate by this mechanism could produce an oxyanion nucleophile analogous

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that seen with chloramphenicol, but one which is incorrectly positioned to attack the thioester carbonyl of acetyl-CoA, accounting for the observed failure of C to acetylate fusidate.

PMID: 7500366 [PubMed - indexed for MEDLINE]

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## Directed evolution of the surface chemistry of the reporter enzyme beta-glucuronidase.

Matsumura I, Wallingford JB, Surana NK, Vize PD, Ellington AD.

Institute of Cellular and Molecular Biology, ICMB A4800/MBB 3.424, Univ of Texas, Austin 78712, USA.

The use of the *Escherichia coli* enzyme beta-glucuronidase (GUS) as a reporter gene expression studies is limited due to loss of activity during tissue fixation with glutaraldehyde or formaldehyde. We have directed the evolution of a GUS variant that is significantly more resistant to both glutaraldehyde and formaldehyde than the wild-type enzyme. A variant with eight amino acid changes was isolated after three rounds of mutation, DNA shuffling, and screening. Surprisingly, although glutaraldehyde is known to modify and cross-link free amines, only one lysine residue was mutated. Instead, amino acid changes generally occurred near conserved lysines, implying that the surface chemistry of the enzyme was selected to either accept or avoid glutaraldehyde modifications that would normally have inhibited function. We have shown that the GUS variant can be used to trace lineages in *Xenopus* embryos under standard fixation conditions, allowing detection of staining when used in conjunction with other reporters.

PMID: 10404164 [PubMed - indexed for MEDLINE]

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